Biochimica et Biophysica Acta 1837 (2014) e105–e117

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

S10 – Membrane transporters, ion pumps and channels

S10.L1

A two-domain elevator mechanism for sodium/proton antiport

Chiara Lee[a], Hae Joo Kang[a], Christoph von Ballmoos[b], Simon Newstead[a], Povilas Uzdavinys[b], David L. Dotson[c], So Iwata[a,d,e], Oliver Beckstein[c], Alexander D. Cameron[a,d,e,f], David Drew[a,b]

[a]*Division of Molecular Biosciences, Imperial College London, London SW7 2AZ, UK*

[b]*Centre for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm University, SE-106 91 Stockholm, Sweden*

[c]*Center for Biological Physics, Department of Physics, Arizona State University, Tempe, AZ 85287-1504, USA*

[d]*Membrane Protein Laboratory, Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Chilton, Oxfordshire OX11 0DE, UK*

[e]*Research Complex at Harwell Rutherford, Appleton Laboratory, Harwell, Oxford, Didcot, Oxfordshire OX11 0FA, UK*

[f]*School of Life Sciences, University of Warwick, Gibbet Hill Road, Coventry CV4 7AL, UK*

E-mail: ddrew@dbb.su.se

Sodium/proton (Na^+/H^+) antiporters, located at the plasma membrane in every cell, are vital for cell homeostasis. In humans, their dysfunction has been linked to diseases, such as, hypertension, heart failure and epilepsy and they are well-established drug targets. The best understood model system for Na^+/H^+ antiport is NhaA from *Escherichia coli*, where both EM and crystal structures are available. NhaA is made up of two distinct domains, a core domain and a dimerisation domain. In the NhaA crystal structure a cavity is located between the two domains providing access to the ion-binding site from the inward-facing surface of the protein. To date, the only reported NhaA crystal structure is of the low pH inactivated form. Here, I will describe the active-state structure of a Na^+/H^+ antiporter, NapA from *Thermus thermophilus* at 3 Å resolution, solved from crystals grown at pH 7.8. In the NapA structure, the core and dimerisation domains are in different positions to those seen in NhaA and a negatively charged cavity has now opened to the outside. The extracellular cavity allows access to a strictly conserved aspartate residue thought to directly coordinate ion-binding, a role supported here by molecular dynamics simulations. To alternate access to this ion-binding site, however, requires a surprisingly large rotation of the core domain, some 20° against the dimerisation interface. We conclude that despite their fast transport rates, Na^+/H^+ antiporters operate by a two-domain rocking bundle model, revealing themes relevant to secondary-active transporters in general.

doi:10.1016/j.bbabio.2014.05.250

S10.L2

Gating in channelrhodopsin

Víctor A. Lórenz-Fonfría[a], Tom Resler[a], Nils Krause[b], Christopher Engelhard[c], Robert Bittl[c], Mirka Neumann-Verhoefen[d], Josef Wachtveitl[d], Christian Bamann[e], Ernst Bamberg[e], Ramona Schlesinger[b], Joachim Heberle[a]

[a]*Exp. Molecular Biophysics, Germany*

[b]*Genetic Biophysics, Germany*

[c]*Electron Spin Resonance and Single Molecule Spectroscopy, Freie Universität Berlin, Germany*

[d]*Institute of Physical and Theoretical Chemistry, Johann Wolfgang Goethe-University, Germany*

[e]*Max Planck Institute of Biophysics, Frankfurt, Germany*

E-mail: joachim.heberle@fu-berlin.de

The discovery of the light-gated ion channel channelrhodopsin (ChR) sets the stage for the novel field of optogenetics where cellular processes are controlled by light. Despite the fact that the crystallographic structure of a ChR chimeric construct was solved (1), the underlying molecular mechanism of light-induced cation permeation in ChR2 remains unknown. We have traced the structural changes of ChR2 by time-resolved IR spectroscopy, complemented by electrophysiological measurements (2,3). The vibrational changes were resolved across the entire chemical time range (10^{-14}–10^{1} s) including the open states of the channel (P_2^{390} and P_3^{520}). Analysis of the amide I vibrations suggests a transient increase in hydration of transmembrane α-helices with $\tau_{1/2} = 60$ µs which tally the onset of cation permeation. We characterized crucial proton transfer steps and found that aspartate 253 accepts the proton released by the Schiff base ($\tau_{1/2} = 10$ µs), the latter being reprotonated by aspartic acid 156 ($\tau_{1/2} = 2$ ms). The internal proton acceptor and donor groups, corresponding to D212 and D115 in bacteriorhodopsin, are clearly different to other microbial rhodopsins indicating that their spatial position in the protein was relocated during evolution. To address structural changes of the channel, ChR2 was subjected to pulsed electron double resonance (pELDOR) spectroscopy (4). Comparison of spin–spin distances in the dark state and after illumination reflect conformational changes in the conductive P_3^{520} state involving helices B and F.

References

[1] H.E. Kato, F. Zhang, et al., Nature 482 (2012) 369–374.
[2] Lórenz-Fonfría, V.A., Resler, et al., Proc. Natl. Acad. Sci. USA 110 (2013), E1273–E1281.
[3] M.K. Neumann-Verhoefen, K. Neumann, et al., J. Am. Chem. Soc. 135 (2013) 6968–6976.
[4] N. Krause, C. Engelhard, et al., FEBS Lett. 587 (2013) 3309–3313.

doi:10.1016/j.bbabio.2014.05.251